



# Ozonation of bezafibrate promoted by carbon materials



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## ABSTRACT

Two carbon materials (multi-walled carbon nanotubes, MWCNT, and activated carbon, AC) were investigated as ozonation catalysts for the mineralization of bezafibrate (BZF) in water. For comparative purposes, kinetic results obtained in the absence of catalyst (single ozonation) and adsorption experiments were also presented. Removal of BZF in the presence of MWCNT is mainly due to a catalytic process, contrarily to what occurs in the presence of activated carbon, which acts mainly as adsorbent. 3-[(4-chlorophenyl)formamido]propanoic acid (BBR), 4-chlorobenzoic acid (pCBA) and 4-chloro-N-[2-(4-hydroxyphenyl)ethyl]benzamide (BBV) were detected as primary products of single and catalytic ozonation of BZF, whereas oxalic, pyruvic and oxamic acids were identified as refractory final oxidation products. The original chlorine of BZF is completely converted to chloride ion and part of nitrogen is converted to  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ . The presence of the radical scavenger *tert*-butanol during catalytic and single ozonation evidenced the participation of  $\text{HO}^\bullet$  radicals in the oxidation process, especially in the mineralization of several intermediates. Microtox tests revealed that simultaneous use of ozone and AC originated lower acute toxicity, which may be due to the contribution of adsorption for the removal from the solution of BZF and its oxidation compounds. The time course of all detected compounds is studied and, as a result, the transformation pathway for the complete mineralization of BZF by single and catalytic ozonation in the presence of the selected carbon materials is also elucidated. Successive experimental runs of BZF degradation carried out with MWCNT show that the catalyst suffers some deactivation as a result of the introduction of oxygenated surface groups on the surface.

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## 1. Introduction

Water contamination by pharmaceuticals represents a rising environmental concern. Bezafibrate (BZF) (2-[4-2-[4-chlorobenzamido]ethylphenoxy]-2-methylpropanoic acid) is a lipidic regulator largely used for the treatment of hyperlipidaemia. This compound is extensively used throughout the world and consequently has been frequently detected in the environment [1,2]. In Germany, BZF has been found in sewage treatment plant (STP) effluents in concentrations up to  $4.6 \mu\text{g L}^{-1}$  and median values of  $2.2 \mu\text{g L}^{-1}$  [1,3]. BZF has been found in rivers and water streams of Germany at median concentrations of  $0.35 \mu\text{g L}^{-1}$  [3], whereas concentrations up to  $57.15 \mu\text{g L}^{-1}$  have been found in Italian rivers [4]. In surface waters this drug has been identified at median concentrations of  $3.1 \mu\text{g L}^{-1}$  [1].

Current wastewater treatment techniques do not efficiently remove pharmaceutically active compounds. Specifically,

compounds of the fibrate family, where BZF is included, are refractory to biological treatment processes [5]. Nanofiltration and reverse osmosis processes appear to be effective methods for the removal of these compounds from drinking water [1,3,6,7], but biofouling of the membrane elements and energy consumption limit the applicability of both processes. Therefore, alternative water treatments, like advanced oxidation methods, have been developed.

The abatement of BZF from water has been investigated using different advanced oxidation processes (AOPs), such as photo-Fenton [8], photo degradation using solar-simulated irradiation [9], ozonation [10] and  $\text{UV}/\text{H}_2\text{O}_2$  [11]. An advanced oxidation/reduction process (AO/RP) using free radical reactions to directly degrade chemical contaminants, like BZF, has also been studied [5].

Catalytic ozonation has emerged as a powerful technology for the treatment of pollutants in water, even for refractory compounds. Some studies have shown that activated carbon (AC) and multi-walled carbon nanotubes (MWCNT) are efficient ozonation catalysts [12,13]. The BZF removal by single ozonation has been reported in the literature [10], whereas no results were found reporting the simultaneous use of ozone and carbon materials. Therefore, the present work aims to study the degradation of

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aqueous solutions of BZF by catalytic ozonation, using carbon materials as catalysts. Several oxidation intermediates and by-products were detected by high performance liquid chromatography (HPLC) and ion chromatography. The time course of all detected compounds was studied and, as a result, the transformation pathways were elucidated.

## 2. Materials and methods

### 2.1. Chemicals

Bezafibrate ( $\geq 98\%$ ) was obtained from Sigma Aldrich and was used as received. 3-[(4-chlorophenyl)formamido]propanoic acid (BBR) (95%) and 4-chloro-N-[2-(4-hydroxyphenyl)ethyl]benzamide (BBV) (95%) were obtained from Ukrorgsyntez Ltd (UORSY). Oxalic ( $\geq 99\%$ ), oxamic ( $\geq 96\%$ ), pyruvic ( $\geq 98\%$ ) and 4-chlorobenzoic (pCBA) (99%) acids, also identified as products of BZF oxidation, were obtained from Sigma Aldrich. *tert*-Butanol ( $\geq 99.7\%$ ), used as  $\text{OH}^\bullet$  radical scavenger, was also obtained from Sigma Aldrich. Acetonitrile ( $\geq 99.9\%$ ), used as eluent in HPLC analyses, was purchased from VWR International (Pro-labo), and ammonium acetate ( $\geq 99\%$ ) and sulphuric acid (95–98%) were obtained from Sigma Aldrich. All solutions were prepared with ultrapure water with a resistivity of  $18.2 \text{ m}\Omega \text{ cm}$  at room temperature, obtained from a Millipore Milli-Q system.

### 2.2. Catalysts characterization

A commercial activated carbon, Norit GAC 1240 PLUS (sample AC), and commercial multi-walled carbon nanotubes, Nanocyl 3100 (sample MWCNT), were used to investigate the catalytic ozonation of bezafibrate. The textural characterization of the materials was based on  $\text{N}_2$  adsorption isotherms determined at 77 K. The surface chemistry of the samples was characterized by the determination of  $\text{pH}_{\text{PZC}}$  (pH at the point of zero charge) and by temperature-programmed desorption (TPD), as described elsewhere [14,15].

### 2.3. Kinetic experiments

The ozonation experiments were carried out in a laboratory scale reactor (ca. 1 L) equipped with agitation and a circulation jacket. Ozone was produced from pure oxygen in a BMT 802X ozone generator. The concentration of ozone in the gas phase was monitored with a BMT 964 ozone analyser. Ozone leaving the reactor was removed in a series of gas washing bottles filled with potassium iodide (KI) solution.

In each ozonation experiment the reactor was filled with 700 mL of a bezafibrate solution with a concentration of  $20 \text{ mg L}^{-1}$ , at the natural pH (around 4.4). In catalytic ozonation experiments, 100 mg of catalyst was introduced in the reactor. The experiments were performed at constant flow rate ( $150 \text{ cm}^3 \text{ min}^{-1}$ ) and constant inlet ozone concentration ( $50 \text{ g m}^{-3}$ ). The stirring rate was maintained constant at 200 rpm, in order to keep the reactor content perfectly mixed. For comparative purposes, both adsorption on carbon materials and single ozonation experiments were performed in the same system, under identical experimental conditions. In the experiments carried out in the presence of *tert*-butanol, a concentration of 0.5 mM of this radical scavenger was used (concentration ten times higher than the initial BZF concentration) [16]. In cyclic experiments, the same procedure was followed. After each experiment, the solution was filtered and the MWCNT sample dried in order to be used in another run. This procedure was repeated two times. Textural and surface chemistry characterization of the MWCNT sample was carried out after the first and the third runs. All experiments were performed at room pressure and

temperature. Samples for analysis were collected at selected times using a syringe and centrifuged. Most of the experiments were carried out in duplicate and the average deviation obtained was  $\pm 2\%$ .

### 2.4. Analytical methods

Concentrations of BZF and reaction products were followed by HPLC using a Hitachi Elite LaChrom HPLC equipped with a diode array detector. For BZF and other compounds the stationary phase used was a LiChroCART Purospher STAR RP-18 ( $250 \text{ mm} \times 4.6 \text{ mm}$ ,  $5 \mu\text{m}$ ) working at room temperature (stationary phase A). These compounds were analyzed under isocratic elution with acetonitrile and ammonium acetate (10 mM, pH 4.0) in the proportion 50:50 v/v and at a flow rate of  $1 \text{ mL min}^{-1}$ , using an injection volume of  $50 \mu\text{L}$ . The retention time for BZF and intermediates A1 (BBR), A2, A3, A4 (pCBA), A5 and A6 (BBV) were 5.8, 3.4, 3.6, 4.1, 4.8, 6.2 and 6.9 min, respectively. The wavelength used for the quantitative measurement of BZF and the detected compounds was 230 nm. Concentrations of some organic acids resulting from the BZF degradation were followed by HPLC using a Hitachi Elite LaChrom HPLC equipped with a UV detector. The stationary phase used was an Alltech OA-1000 column ( $300 \text{ mm} \times 6.5 \text{ mm}$ ), working at room temperature (stationary phase B), under isocratic elution with a solution of  $\text{H}_2\text{SO}_4$  5 mM at a flow rate of  $0.5 \text{ mL min}^{-1}$  and using an injection volume of  $15 \mu\text{L}$ . All compounds were measured at 200 nm. The retention time for by-products B1, B2, B3 (oxalic acid), B4, B5, B6, B7 (pyruvic acid), B8 (oxamic acid) and B9 were 5.4, 5.6, 5.8, 6.8, 7.4, 7.9, 8.1, 8.7, 9.3 min, respectively.

The concentration of inorganic ions released during ozonation experiments was determined by ion chromatography. The anions (nitrite, nitrate and chloride) were identified and quantified in a Dionex ICS-2100 Ion Chromatography System using a Dionex Ion-Pac AS11-HC column ( $250 \text{ mm} \times 4 \text{ mm}$ ), under an isocratic elution with a solution of NaOH 30 mM at a flow rate of  $1.5 \text{ mL min}^{-1}$ . A sample loop of  $10 \mu\text{L}$  was used for analysis, at  $30^\circ\text{C}$ . In such conditions, the retention times for chloride, nitrite and nitrate were 2.9, 3.3 and 5.2 min, respectively. For the determination of ammonium, an IonPac CS12A column ( $250 \text{ mm} \times 4 \text{ mm}$ ) was used in a Dionex DX-120 Ion Chromatography System, working with a solution of methanesulfonic acid 20 mM as the mobile phase at a flow rate of  $1.0 \text{ mL min}^{-1}$ . In such conditions, the retention time was 4.1 min. The analyses were carried out at room temperature using an injection volume of  $25 \mu\text{L}$ . The system was equipped with a conductivity detector, which performance was improved by electrolyte suppression using ASRS 300 or CSRS ULTRA II self-regeneration suppressors for anions and cations, respectively.

The degree of mineralization of BZF solution was obtained by Total Organic Carbon (TOC) determination in a Shimadzu TOC-5000A analyser.

Microtox acute toxicity tests were performed in order to assess the acute toxicity in natural media caused by compounds produced during the BZF ozonation. These experiments were carried out using the Microtox basic test (Azure Environmental, USA), according to the procedure described in the standard ISO/DIS 11348-3 [17]. The special light-emitting bacterial reagent (*Vibrio fischeri*) was purchased from Hach Lange. This test measures the inhibition of the light emission of bioluminescent bacteria (*Vibrio fischeri*) caused by the toxic effect of the tested chemicals, during an incubation period of 30 min at  $15^\circ\text{C}$ . Results were corrected by time dependent change in light emission under test conditions without any toxic influence and the percentage difference between initial and final light output was quantified. For each sample, analyses were performed in duplicate.

### 3. Results and discussion

#### 3.1. Characterization results

The differences in the textural properties between AC and MWCNT are evident. In fact, AC presents a much larger specific surface area than MWCNT ( $S_{\text{BET}} = 809$  and  $331 \text{ m}^2 \text{ g}^{-1}$ , respectively); however, comparing mesopore surface areas, sample MWCNT presents a value two times higher than AC ( $S_{\text{meso}} = 331$  and  $158 \text{ m}^2 \text{ g}^{-1}$ , respectively), indicating that AC is essentially microporous ( $V_{\text{micro}} = 0.338 \text{ cm}^3 \text{ g}^{-1}$ ). These observations were expected because multi-walled carbon nanotubes and activated carbon present clear differences in their structure. Activated carbons are generally described as a group of randomly cross-linked aromatic sheets and strips, with variable gaps between them, corresponding to the pores of the material [18]. In comparison, ideal MWCNT may be described as nanoscale graphene cylinders that are closed at each end by half a fullerene [19], although the occurrence of defects is common both at the tube ends and on the sidewalls [20]. Concerning the surface chemistry, both materials present a small amount of oxygen-containing surface groups (only about 1 wt% oxygen on the surface) and it is noticed that AC presents a slightly basic character, while MWCNTs are neutral ( $\text{pH}_{\text{PZC}}$  values of 8.5 and 7.0, respectively).

#### 3.2. Catalytic ozonation of bezafibrate

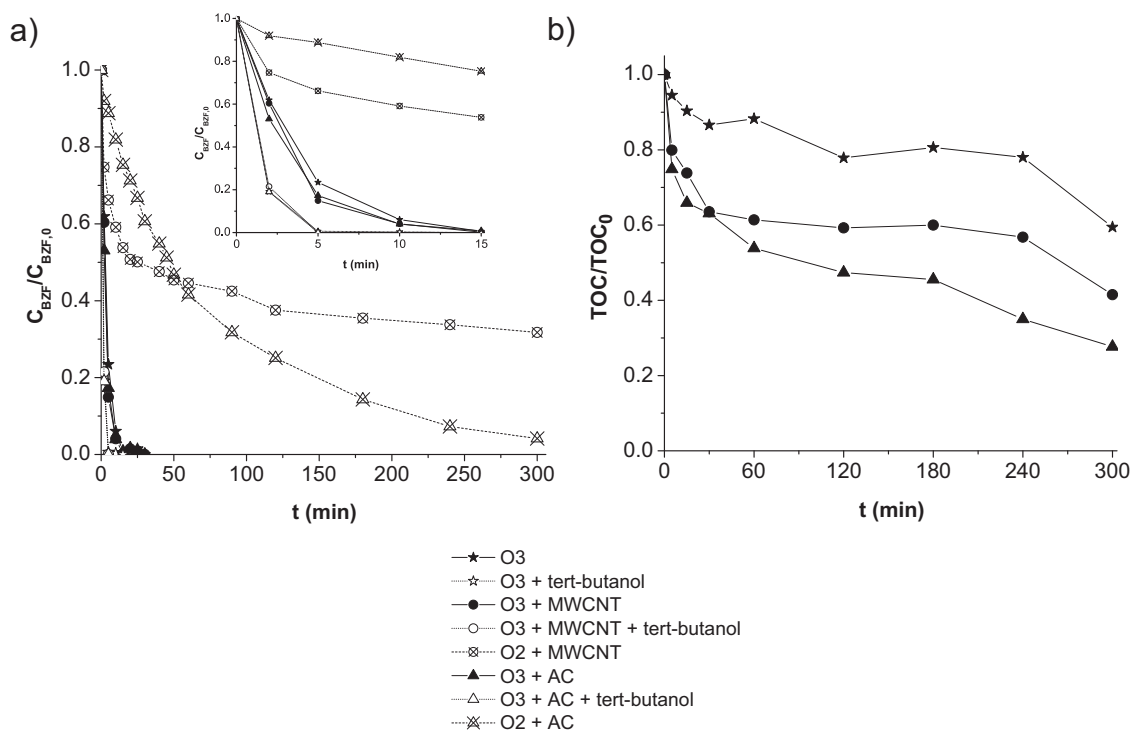
##### 3.2.1. Bezafibrate degradation

Samples MWCNT and AC were tested as catalysts in the ozonation of a BZF solution at natural pH (around 4.4). In addition to catalytic ozonation, ozonation in the absence of the catalyst (single ozonation) was also carried out. In order to evaluate the adsorption capacity of the catalysts towards BZF, adsorption experiments were performed. With the purpose of investigating the mechanism, experiments in the presence of the radical scavenger *tert*-butanol were also carried out. All experiments were followed during 5 h.

The experimental results corresponding to bezafibrate and TOC decay are depicted in Fig. 1. Ozonation by itself enables a fast decay of BZF concentration and the removal of this compound is achieved in less than 20 min. This is observed because ozone selectively attacks activated aromatic rings, which are present in BZF. There are no significant differences between single ozonation and catalytic ozonation. However, when TOC results are compared, the presence of both catalysts leads to higher mineralization degrees than single ozonation. With the simultaneous use of ozone and MWCNT, the TOC removal after 300 min is approximately 60%, whereas in the presence of AC a removal of 73% was achieved.

Concerning the adsorption results, activated carbon adsorbs larger amounts of BZF than MWCNT. In fact, the adsorption experiment on AC resulted in the removal of almost all the amount of BZF after 300 min (more than 95%), compared to 70% with MWCNT. These different performances are justified by the large difference in the textural properties of the materials. In the first minutes the adsorption is faster in the MWCNT sample, due to lower mass transfer limitation in its mesoporous structure in comparison with the microporous AC, but higher uptakes were obtained for long contact times for AC due to its larger surface area. In the case of activated carbon, adsorption even leads to a higher TOC removal than ozonation, which is explained by the easier adsorption of the BZF molecule than the respective oxidation products.

Fig. 1 also shows that the presence of the radical scavenger *tert*-butanol during catalytic and single ozonation accelerates the BZF degradation, indicating that the scavenging of the radicals in solution favours the reaction between BZF and ozone, which is more effective than radicals for the degradation of BZF. Nevertheless, as mentioned above, the main advantage of using a catalyst in the ozonation of BZF is the mineralization of solutions. Unfortunately, due to the important contribution of *tert*-butanol to the TOC value of the solution, it was not possible to follow this parameter in this type of experiments.



**Fig. 1.** Evolution of the dimensionless (a) BZF and (b) TOC concentrations at natural pH ( $\sim 4.4$ ) during adsorption, catalytic and non-catalytic ozonation and effect of *tert*-butanol ( $C_{0,\text{BZF}} = 20 \text{ mg L}^{-1}$ ,  $C_{0,\text{tert-butanol}} = 0.5 \text{ mM}$ , catalysts  $= 0.14 \text{ g L}^{-1}$ ).

**Table 1**

Intermediates (A1, A4, A6) and by-products (B3, B7, B8) identified during single and catalytic ozonation of BZF.

Name	Compound
A1	3-[(4-Chlorophenyl)formamido]propanoic acid (BBR)
A4	4-Chlorobenzoic acid (pCBA)
A6	4-Chloro-N-[2-(4-hydroxyphenyl)ethyl]benzamide (BBV)
B3	Oxalic acid
B7	Pyruvic acid
B8	Oxamic acid

### 3.2.2. Cyclic experiments

Reutilization of the MWCNT sample was carried out with the purpose of studying the influence of ozonation on its surface chemistry, and the eventual deactivation during mineralization of BZF. The kinetic results obtained are depicted in Fig. 2. By comparing the curves of BZF removal (Fig. 2a), no significant differences in the decay of BZF concentration were observed during reutilization of the catalyst. This was expected because BZF is easily oxidized by ozone, as it was explained before. However, when TOC results are compared (Fig. 2b), a slight decrease in the mineralization level is observed, being particularly evident from the first to the second run. It can be observed that the curves obtained for the TOC removal seem tending to the curve of a MWCNT sample oxidized with nitric acid (MWCNT-HNO<sub>3</sub>), which has high amounts of oxygenated groups on the surface [13]. Hence, even after three cycles, MWCNT continues to be active in the ozonation of BZF.

TPD spectra of the samples before and after the first and the third run (see Fig. 3) show an increase in the amount of oxygen-containing surface groups in the reused catalyst. This confirms that the MWCNT sample is, in fact, oxidized by dissolved ozone, according to what was obtained during oxalic acid and sulfamethoxazole removal in previous studies [13]. The observed decrease in the activity in successive runs is due to the introduction of oxygenated electron-withdrawing groups, which reduce the electron density on the carbon surface, thus decreasing the catalytic activity of the material for the decomposition of ozone into hydroxyl radicals [13], which are more efficient in mineralization of organic compounds than ozone. After the third run, significant peaks are observed at approximately 350 °C in both CO and CO<sub>2</sub> spectra and at high temperatures in the CO spectrum, which may result from the decomposition of organic compounds adsorbed on the MWCNT surface [14,15]. The surface area of MWCNT increases after successive runs (from 290 m<sup>2</sup> g<sup>-1</sup> before ozonation to 345 m<sup>2</sup> g<sup>-1</sup> after third run), meaning that the oxidation by dissolved ozone promotes a weak change in the textural properties of the MWCNT.

### 3.2.3. Identification and analysis of oxidation intermediates and by-products

As a result of BZF oxidation in the presence of ozone several intermediates and by-products are formed. Identification of the most relevant oxidation products is a fundamental task to predict the environmental impact of the original compound (BZF). So, the studies described in the previous section were accompanied by the identification and quantification of reaction intermediates and by-products: aromatic compounds and aliphatic carboxylic acids. Thus, the collected samples were analyzed by HPLC using different stationary phases: a reversed-phase C18 column (A) to detect aromatic compounds and an ion-exclusion column (B) to analyse organic acids, as thoroughly explained in the experimental section.

Following the methodology described in a previous work [21], six degradation intermediates and by-products were identified, as shown in Table 1. The evolutions of all detected compounds, resulting from the ozonation of BZF, including in the presence of *tert*-butanol, are presented in the supplementary data (Figs. S1 and S2).

It was concluded that all compounds detected in stationary phase A (A1–A6) are produced from the beginning of reaction (the first sample collected). Compound A2 is produced in higher extension and disappears earlier when *tert*-butanol is used, indicating that it and its organic parent compounds have aromatic rings and/or unsaturated bonds, since they are more easily formed and degraded by ozone than by HO• radicals. Compounds A1 (BBR), A4 (pCBA), A3, B5, B7 (pyruvic acid) and B9 are early produced in larger amounts and do not disappear or disappear much later in the experiments with *tert*-butanol, which is indicative that they are resulting mainly from the direct oxidation by ozone of their parent compounds and/or the hydroxyl radicals are the main oxidant involved in their degradation mechanism. B2, B4 and B8 (oxamic acid) are mainly produced by the radical mechanism because they are formed in lower amounts when *tert*-butanol is present in solution, where only molecular ozone was available to react with their parent intermediates. A5 molecule, as well as its precursor molecule(s), should not contain aromatic rings or unsaturated bonds, since they are produced from molecule(s) that is (are) more easily degraded by hydroxyl radicals than by ozone. A6 (BBV) and B6 are only produced in the absence of *tert*-butanol, which suggest that these compounds are resultant from BZF decomposition by a radical mechanism.

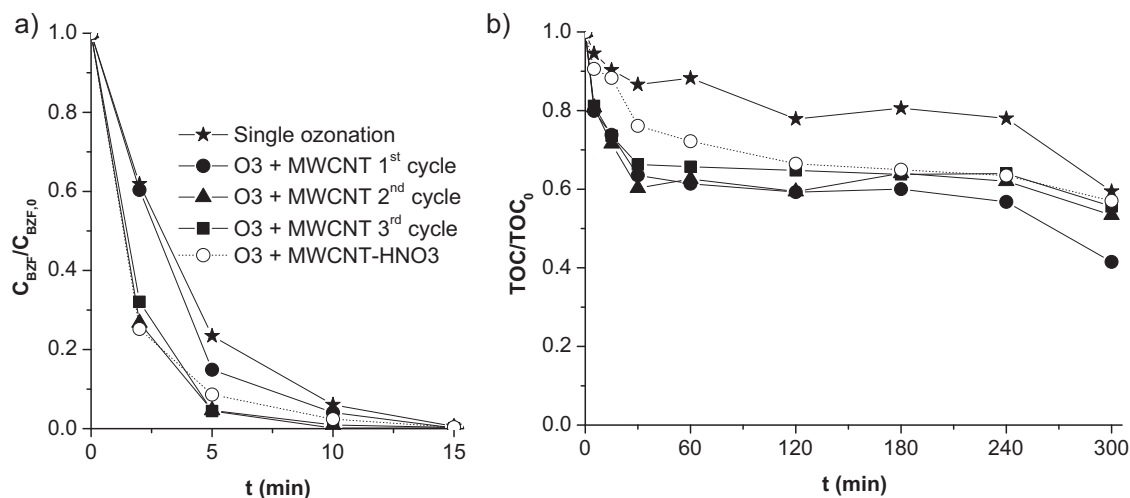
Concerning compounds detected in the stationary phase A, no significant differences between single and catalytic ozonation in absence of *tert*-butanol in their appearance/disappearance were observed. However, important differences were obtained in the evolution profiles of some of the organic acids. This is particularly evident with B3 (oxalic acid) and B4, which are produced in much higher extension in the absence of catalyst, indicating that its presence, mainly MWCNT, leads to higher conversions of these compounds. It is also important to mention the accumulation of oxamic acid observed both with and without catalyst, suggesting the presence in solution of intermediates containing N. Catalytic ozonation contributes to higher accumulation of oxamic acid (mainly in the presence of MWCNT) than single ozonation, which suggests that the former process is more efficient in the degradation of intermediates containing N. By-product B6 accumulated during the 5 h experiment of single ozonation, whereas in the presence of catalyst its concentration achieved a maximum, indicating a higher ability for degradation.

These observations indicate an improvement of the mineralization degree of BZF when a catalyst and ozone are simultaneously used, as well as a better catalytic performance of MWCNT.

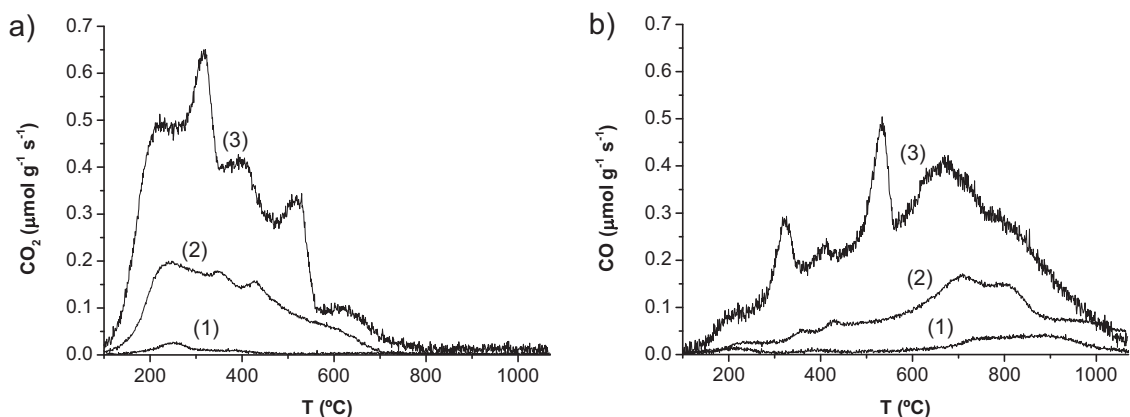
The evolutions of all detected intermediates and by-products for ozonation in the presence of MWCNT are depicted in Fig. 4. For all ozonation experiments, this information is depicted in the supplementary data (Fig. S3), since the trend is similar. The evolution is presented as areas obtained in chromatograms as a function of reaction time. Evolution of all detected compounds in column A is shown in Fig. 4a. For both single and catalytic ozonation the evolution of these compounds is similar, i.e. they are mainly produced during the initial stage of experiments and are easily removed from solution (practically all of them were not detected after 30 min). Evolution of the detected acids is depicted in Fig. 4b. In general, only compounds B5 and B9 do not persist in solution after 300 min of single or catalytic ozonation, whereas concentrations of compounds B1 and B7 decrease, suggesting their complete removal for longer times. However, concentrations of some by-products, such as B2 and B8 (oxamic acid), continuously increase during experiments, which suggests the existence of intermediates that are still being oxidized.

For each ozonation experiment, the values of TOC removed and present in solution (both non-identified and identified as compounds) were calculated after 5, 30, 180 and 300 min of reaction. These results are shown in Table 2. The fraction of TOC identified was determined based on the obtained concentrations of





**Fig. 2.** Evolution of the dimensionless (a) BZF and (b) TOC concentrations at natural pH ( $\sim 4.4$ ) during catalytic cyclic ozonation experiments ( $C_{0,BZF} = 20 \text{ mg L}^{-1}$ , catalyst =  $0.14 \text{ g L}^{-1}$ ).



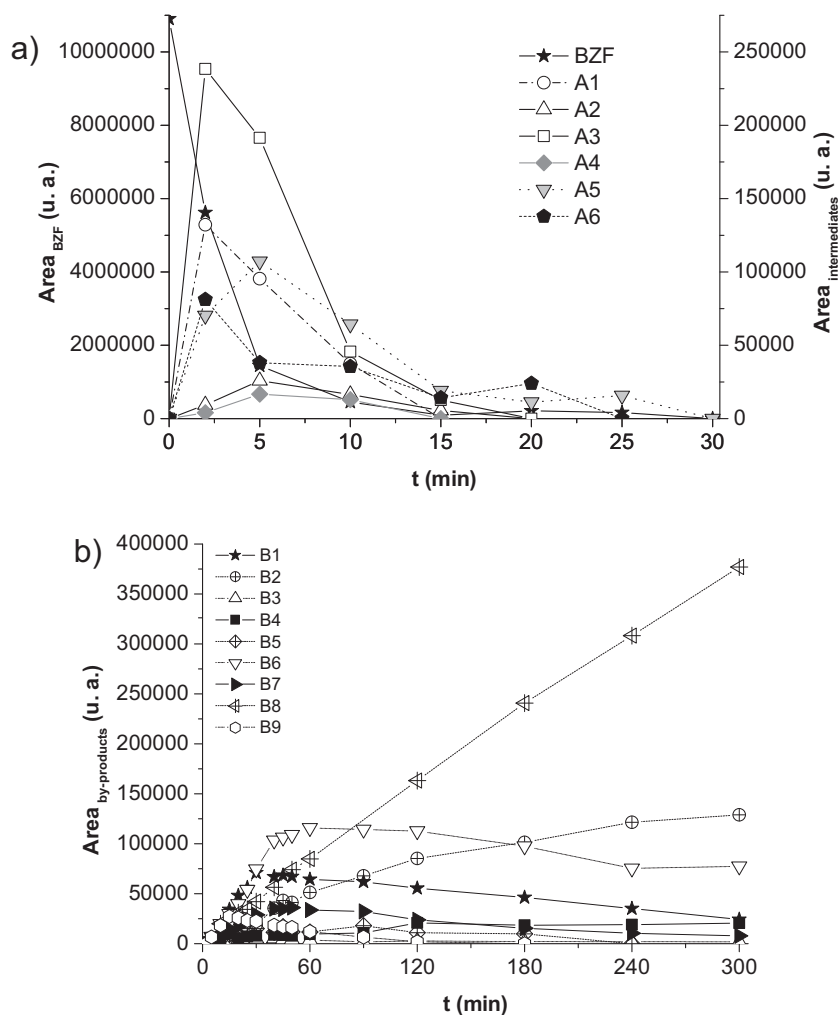
**Fig. 3.** TPD spectra for samples used in cyclic experiments: (a)  $\text{CO}_2$  evolution; (b)  $\text{CO}$  evolution; (1) MWCNT, (2) MWCNT after first run, (3) MWCNT after third run.

**Table 2**  
TOC removed and present (both non-identified and identified as compounds) achieved after 5, 30, 180 and 300 min of single and catalytic ozonation of BZF.

Experiments		TOC <sub>removed</sub> (%)	TOC <sub>non-identified</sub> (%)	TOC <sub>identified</sub> (%)	$\frac{\text{TOC}_{\text{non-identified}}}{\text{TOC}_{\text{identified}}}$	Contribution for TOC of each compound identified (%)					
						BBR	pCBA	BBV	Oxalic acid	Pyruvic acid	Oxamic acid
5 min	$\text{O}_3$	5.5	91.1	3.4	27.0	0.73	0.11	1.40	0.54	0.51	0.09
	$\text{O}_3 + \text{MWCNT}$	20.1	78.0	1.9	40.3	0.65	0.04	0.83	0	0.31	0.09
	$\text{O}_3 + \text{AC}$	25.2	72.5	2.3	30.8	0.76	0.03	1.12	0	0.32	0.12
30 min	$\text{O}_3$	13.4	82.9	3.7	22.6	0	0	0	1.27	2.23	0.18
	$\text{O}_3 + \text{MWCNT}$	36.5	60.9	2.6	23.2	0	0	0	0.63	1.63	0.35
	$\text{O}_3 + \text{AC}$	36.9	61.0	2.1	29.1	0	0	0	0.69	1.05	0.36
180 min	$\text{O}_3$	19.4	76.3	4.3	17.7	0	0	0	3.00	0.47	0.84
	$\text{O}_3 + \text{MWCNT}$	40.0	56.8	3.2	17.5	0	0	0	0.58	0.92	1.74
	$\text{O}_3 + \text{AC}$	54.5	42.7	2.8	15.3	0	0	0	0.68	0.79	1.32
300 min	$\text{O}_3$	40.5	54.1	5.4	10.0	0	0	0	3.82	0	1.56
	$\text{O}_3 + \text{MWCNT}$	58.5	37.7	3.8	10.0	0	0	0	0.58	0.52	2.69
	$\text{O}_3 + \text{AC}$	72.3	24.5	3.2	7.8	0	0	0	0.67	0.40	2.07

identified oxidation products, namely three cyclic compounds (A1-BBR, A4-pCBA and A6-BBV) and three carboxylic acids (B3-oxalic, B7-pyruvic and B8-oxamic acids). Table 2 shows that the fraction of TOC not identified as specific compounds is significant. However, that fraction tends to decrease with the reaction time. In the initial stages of BZF ozonation, unsaturated compounds are produced but practically disappear after a few minutes due to their fast reaction with ozone, originating progressively lower molecular weight organic compounds (e.g., ketones, aldehydes and carboxylic acids).

Thus, the organic matter present in the solution after 5 min will be mainly due to unsaturated organic compounds, whereas at 300 min carboxylic acids will have a predominant contribution. In fact, the results presented in Table 2 show a complete removal of the identified primary products (BBR, BBV and pCBA) from 5 to 30 min, as well as a significant increase in the contribution of the identified carboxylic acids from 30 min of reaction. Oxamic and oxalic acids were the compounds with major contributions to the increase of that fraction, showing their high persistence. This suggests that they



**Fig. 4.** Evolution of BZF and all detected intermediates and by-products during BZF catalytic ozonation in the presence of MWCNT at natural pH ( $\sim 4.4$ ): (a) BZF and aromatic compounds, (b) organic acids.

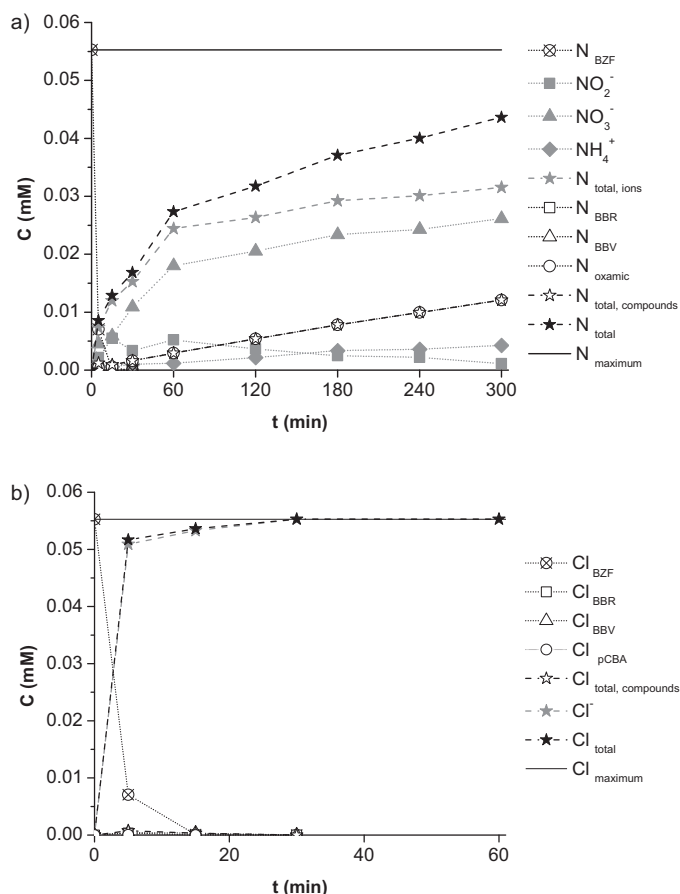
can be formed from different pathways. After 300 min of reaction, ozonation with MWCNT leads to a higher contribution of oxamic acid for the identified TOC than with AC or single ozonation. On the other hand, in single ozonation, the highest contribution for the identified TOC is due to oxalic acid.

The conversion of the initial nitrogen and chlorine contents of BZF to inorganic ions such as nitrate, nitrite, ammonium and chloride was investigated by ion chromatography. The evolution of  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations for ozonation in the presence of MWCNT is presented in Fig. 5a, whereas Fig. 5b shows the evolution of the  $\text{Cl}^-$  concentration. This information was also presented for the remaining experiments in Fig. S4 of the supplementary data, since the trend is similar. The limits for the concentration of N and Cl ( $\sim 0.053$  mM) are also indicated in the figures. The evolution of nitrogen and chloride concentrations presented in the identified products during BZF degradation is also shown.

All chlorine present in BZF is converted into  $\text{Cl}^-$  during the first stages of the ozonation experiments. In fact, the presence of MWCNT during ozonation leads to 100% chlorine conversion to chloride within 30 min, whereas in ozonation with activated carbon and without catalyst the complete conversion is achieved after 60 min and 120 min, respectively. These results indicate that there are no chlorine-containing organic compounds in solution after this ozonation time, suggesting high lability of the chlorine-containing ring present in the BZF molecule.

Some BZF-derived nitrogen is mineralized in the form of nitrite, nitrate and ammonium ions. There is a significant difference between single and catalytic ozonation (see Fig. S5 of supplementary data). After 5 h of single ozonation, only 22% of N was mineralized, whereas in the presence of MWCNT and AC, after the same time period, 52 and 56% of nitrogen, respectively, was converted to inorganic ions. Moreover, ozonation in the absence of catalysts leads to the smallest amount of N-containing oxamic acid (12%), when compared with 22 and 17% obtained in the presence of MWCNT and AC, respectively. This indicates that catalytic ozonation is more efficient in the conversion of initial nitrogen to nitrogenated final oxidation product (like oxamic acid) and inorganic ions than single ozonation. The amount of non-identified nitrogen corresponds to values of about 25% for catalytic ozonation and 65% for single ozonation. The incomplete nitrogen mass balance indicates that other non-identified refractory nitrogenated compounds are formed, contributing to the remaining TOC in the solution.

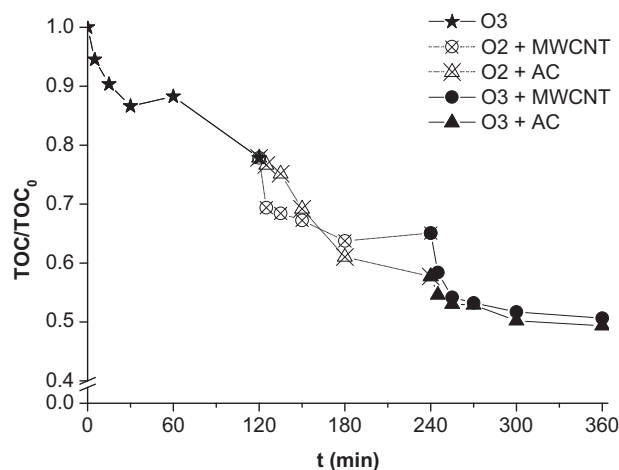
In Fig. 5a and in Fig. S4 of the supplementary data, it is possible to observe the evolution of inorganic ions formed. During the first stages of ozonation experiments, nitrite is produced in higher amount than nitrate and ammonium, but this trend is inverted after a short time (about 30 min). After 5 h, the concentration of  $\text{NO}_2^-$  present can be neglected in comparison with those of the other two ions. The different amounts of these released ions may be linked to the different features of the initial N-containing structure



**Fig. 5.** Evolution of inorganic ions, containing N (a) and Cl (b), during catalytic ozonation of BZF in the presence of MWCNT at natural pH ( $\sim 4.4$ ) ( $C_{0,BZF} = 20 \text{ mg L}^{-1}$ , catalyst =  $0.14 \text{ g L}^{-1}$ ).

[21,22]. The position of the N atom during oxidation by  $\text{OH}^\bullet$  radicals of N-containing molecules may influence the proportion of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  produced, as reported before [21–24]. Thus, according to the obtained results, the single N atom present in the structure of the BZF molecule is susceptible of originating preferentially  $\text{NO}_3^-$ . In the case of single ozonation,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were released in the relative proportion of about 3.1/1, whereas in catalytic ozonation in the presence of either MWCNT or AC those inorganic ions are produced in the proportion of 6.1/1 and 8.3/1, respectively. This observation suggests that the presence of catalysts in BZF ozonation leads to the formation of N-containing organic intermediates that are oxidized predominantly to  $\text{NO}_3^-$ , indicating that the preferential degradation pathway of BZF during ozonation may be influenced by the presence of catalyst.

In order to understand the role of MWCNT and AC and the type of contribution of these materials in the process, a sequential experiment constituting by three steps was performed for each carbon material. In the first step, a solution of BZF ( $C_0 = 20 \text{ mg L}^{-1}$ ) at natural pH was ozonized for 2 h to remove unsaturated compounds produced from primary BZF oxidation. After that, oxygen was bubbled into the solution to remove the dissolved ozone and, subsequently, 100 mg of carbon material was introduced in the reactor; the period of adsorption last 2 h, in order to evaluate the contribution of each carbon material in the adsorption of the refractory molecules, such as carboxylic acids. In the third step, the performance of both materials in the removal of these compounds was assessed by replacing the oxygen stream by an ozone-containing stream during 2 h. Samples from the solution were collected for TOC analysis. The obtained results, presented

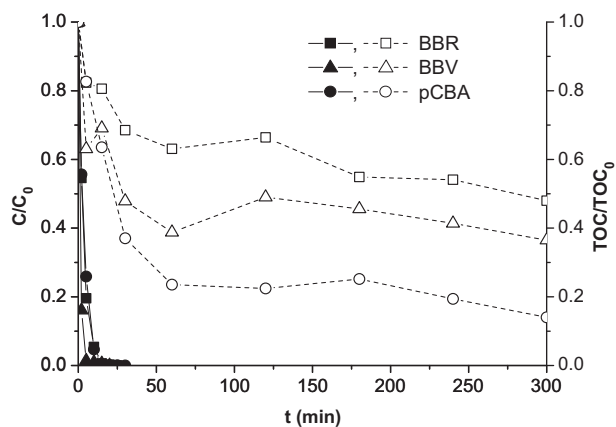


**Fig. 6.** Evolution of dimensionless TOC concentration during sequential experiments at natural pH, starting from a solution with  $20 \text{ mg L}^{-1}$  of BZF: (I) O<sub>3</sub>, (II) O<sub>2</sub> + carbon material, (III) O<sub>3</sub> + carbon material.

in Fig. 6, indicate that adsorption on MWCNT contributes more weakly to the removal of the compounds that result from BZF oxidation (about 10%) compared to catalytic ozonation (15%). Assuming that after 2 h of reaction, the oxidation products remaining in solution are less reactive towards ozone and less prone to adsorption on MWCNT, the increase in TOC removal obtained when ozone and MWCNT are used simultaneously is mainly due to a catalytic effect. On the contrary, adsorption on activated carbon strongly contributes to the removal of the compounds that result from BZF oxidation (20%) compared to catalytic ozonation (8%), which indicates that the increase in TOC removal obtained when ozone and AC are simultaneously used is largely owing to the adsorption on the activated carbon surface. Therefore, it can be concluded that the sample MWCNT presents a higher catalytic performance than activated carbon for the mineralization of BZF, which was justified by their differences in surface chemistry and by the higher internal mass transfer resistances expected for activated carbons (essentially microporous). On the other hand, the adsorption on the surface of activated carbon has a strong contribution to the removal of bezafibrate.

### 3.2.4. Proposed pathways for BZF mineralization

**3.2.4.1. Catalytic ozonation of the identified BZF oxidation products.** In order to better understand the oxidation pathways during BZF ozonation, all identified intermediates and by-products were oxidized by ozone in the presence of the sample MWCNT, using the same system and under identical experimental conditions as in BZF ozonation. The evolution of BBR, pCBA and BBV, as well as the TOC of these solutions is depicted in Fig. 7. In a previous study [21], the ozonation results for oxalic, oxamic and pyruvic acids under the same experimental conditions were reported. BBR, BBV and pCBA are easily degraded by ozone, being completely removed up to 20 min. Nevertheless, after 5 h, only about 52% of mineralization was achieved for BBR and 63% for BBV, whereas for pCBA 86% of mineralization was reached. By analysis of the degradation products of the identified intermediates of BZF oxidation, presented in Fig. S6 of the supplementary data, it can be concluded that BBR ozonation resulted in the formation of compounds A3, A4 (pCBA), A5, whereas BBV originated compounds A1 (BBR), A2, A3 and A4 (pCBA). Both BBR and BBV led to the formation of all organic acids detected (B1–B9). In the case of pCBA, only A3, B1, B3, B4, B7 and B9 were formed. As reported in a previous study [21], the ozonation of oxamic, pyruvic and oxalic acids (B8, B7 and B3, respectively) only gives CO<sub>2</sub>. Oxamic acid is very refractory



**Fig. 7.** Evolution of dimensionless concentration of various BZF degradation products and TOC removal (black and white symbols, respectively) during catalytic ozonation in the presence of MWCNT, at natural pH ( $C_{0, \text{BBR}} = C_{0, \text{BBV}} = C_{0, \text{pCBA}} = 20 \text{ mg L}^{-1}$ , catalyst =  $0.14 \text{ g L}^{-1}$ ).

to ozonation [13]; this explains its accumulation in solution (see Fig. 5a and b).

A degradation sequence for catalytic and single ozonation of BZF was proposed, based on the previous results, as well as on the analysis of the evolution of all intermediates and by-products, during catalytic and non-catalytic ozonation of BZF. This scheme is presented in Fig. S7.

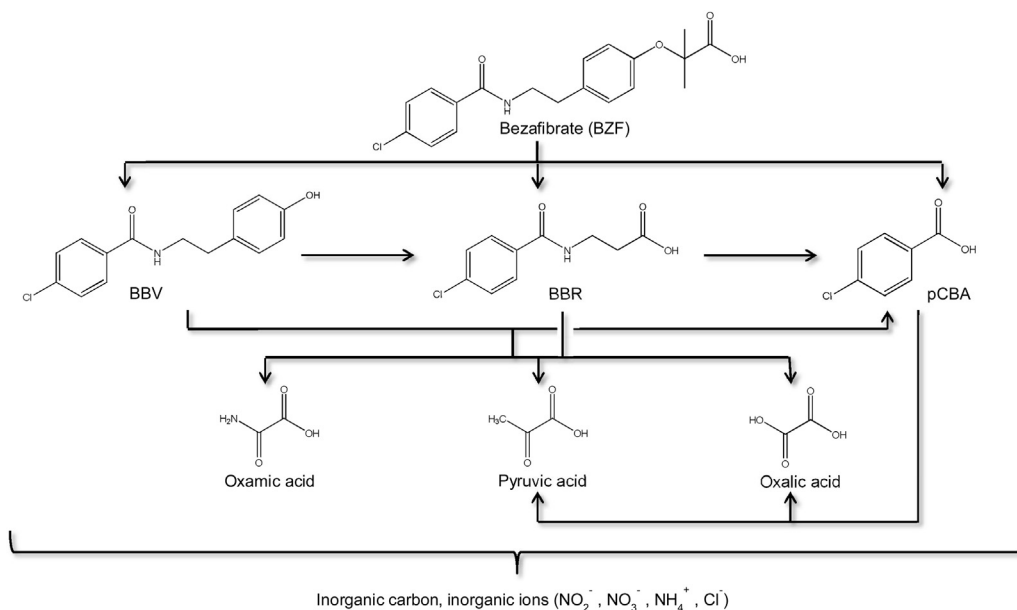
**3.2.4.2. Analysis of the products resulted from BZF oxidation by different AOPs.** Some intermediates resulting from BZF degradation by three advanced oxidation processes, such as photo degradation using solar-simulated irradiation [9], ozonation [10] and UV/H<sub>2</sub>O<sub>2</sub> [11] were identified. In other recent study, which concerned the free-radical-induced oxidative and reductive degradation of BZF, the major degradation products were also reported [5]. None of these works mention any carboxylic acid. In the present work, the formation of the BZF oxidation products reported in those studies was investigated. However, among those compounds, only three were identified as degradation products of BZF by single or catalytic ozonation under the experimental conditions used: BBR

was reported by Dantas et al. [10] in ozonation; BBR was found during photo degradation [9], UV/H<sub>2</sub>O<sub>2</sub> [11] and the mentioned oxidative/reductive treatment [5]; pCBA was identified in UV/H<sub>2</sub>O<sub>2</sub> oxidation [11]. It was not possible to confirm the presence of some compounds identified in the mentioned studies during the ozonation experiments due to the unavailability of the corresponding chemical standards.

**3.2.4.3. Pathways for BZF degradation.** The pH of the medium plays an important role on the reaction mechanism. In the present work, BZF was ozonated at natural pH ( $\sim 4.4$ ), which is higher than pK<sub>a</sub> (3.6) [25], meaning that the corresponding anion is the predominant specie.

It is well known that compounds presenting in their structures double bonds and aromatic rings readily react with ozone. Molecular sites with a strong electronic density are more susceptible to ozone electrophilic attack, like in the case of compounds substituted with electron donor groups (such as -OH and -NH<sub>2</sub>). On the contrary, aromatics substituted with electron withdrawing groups (-COOH) are weakly reactive to ozone. Observing the structure of BZF (Fig. 8), the two aromatic rings represent possible centres of attack. However, Cl present in the BZF molecule is an electron withdrawing group, reducing the electron density and disfavoring the ozone electrophilic attack in the aromatic moiety.

Based on the previous identification of oxidation intermediates, a possible ozonation pathway of BZF is proposed in Fig. 8. The first step consists in the hydroxylation of both aromatic rings of BZF, as suggested by Dantas et al. [10]. The species resultant from the unchlorinated ring-opening could originate the BBR molecule by an anomalous ozonation mechanism [26]. The presence of BBV in solution may be explained by the addition of HO• to the ipso-O position, removing the fibrate chain (2-hydroxyisobutyric acid). However, this molecule was not detected, which suggests that either it is not formed in appreciable amounts or further oxidation prevents its build-up in solution. According to experiments performed in the presence of the radical scavenger, this pathway is only followed by the radical mechanism. When BBV is formed, both aromatic rings of the molecule may suffer hydroxylation reactions, and thus the anomalous ozonation mechanism mentioned above may occur, originating BBR. Another possibility is the scission of the amide group, leading to formation of pCBA. The presence in solution of



**Fig. 8.** Proposed degradation pathways of bezafibrate in single and catalytic ozonation.



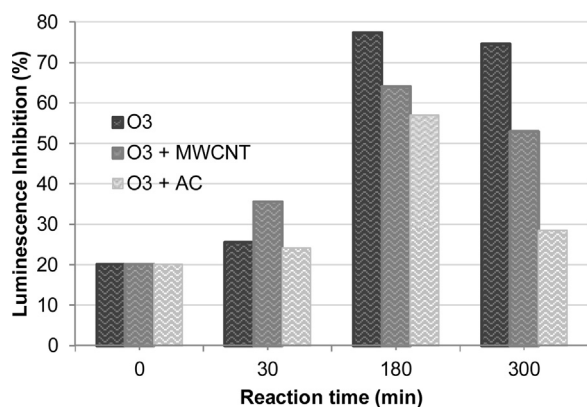


Fig. 9. Results of microtox tests at 0, 30, 180 and 300 min of reaction, with exposure time at bacteria *Vibrio fischeri* of 30 min.

another moiety of BZF that could be originated by this cleavage was not confirmed due to the unavailability of the corresponding chemical standard. This scission may also occur in the degradation of BBR and BBV, since these molecules present the amide group in their structures. In fact, the results obtained from the ozonation of BBV and BBR under selected experimental conditions confirm the presence of pCBA as oxidation product of those compounds (see Fig. S5). Nevertheless, the molecules that would result from scission, like tyramine and  $\beta$ -alanine (resulting from BBV and BBR scission, respectively) were not found. Therefore, these compounds should be further oxidized, preventing their build-up in solution. The production of the identified organic acids has the contribution of different pathways.

The identified reactions occur during ozonation of BZF in all experiments (under the defined conditions), suggesting that BZF is removed by the same pathways, but the predominance of one over the other depends on the catalyst used.

### 3.2.5. Bioassays experiments

During water treatment, compounds more toxic than the target compound may be formed. In order to assess the toxicity caused by compounds produced during single and catalytic ozonation, the acute toxicity of the untreated BZF solution and solutions submitted to ozonation during 30, 180 and 300 min were evaluated by Microtox bioassays. The marine bacteria *Vibrio fischeri* was used to determine the influence of toxic substances on pure bacteria. The results are presented in Fig. 9 and were obtained after 30 min of exposition by calculation of percentage of inhibition in the bacteria luminescence caused by each sample.

The results showed an increase in the inhibition percentage of the bacteria luminescence during the initial stages of ozonation experiments up to 180 min, which means that the intermediates formed in this period, either in the presence or absence of catalyst, have higher acute toxicity than BZF. As aromatic compounds are the major intermediates present in the solution during this period, (confirmed by HPLC), it can be concluded that they are highly toxic. However, in the presence of catalysts, the increase of inhibition is not as significant as in single ozonation, which may be due to the higher amounts of those compounds produced during the reaction in the absence of catalyst. For longer reaction times, the formation of intermediates with lower acute toxicity promotes a decrease in the inhibition percentage of the bacteria luminescence, indicating that the acute toxicity of the treated solutions tend to values close to the BZF untreated solution. This fact is explained by the predominant presence of organic acids in the solution at higher reaction times. The lower toxicity obtained in the catalytic ozonation with AC comparing with the remaining experiments may be due not only to the types and amounts of organic compounds present in solution

but mainly to strong adsorption of bezafibrate and some of the respective oxidation products on the surface of activated carbon, as discussed before.

## 4. Conclusions

In the present work, two carbon materials (MWCNT and AC) were investigated as ozonation catalysts for the mineralization of bezafibrate (BZF) solutions. For comparison purposes, results of ozonation in the absence of catalyst were also obtained.

Ozonation is an efficient process for the removal of bezafibrate from aqueous solution, although leading to the formation of several organic compounds. The removal rate of bezafibrate is not significantly influenced by the presence of catalyst. However, the mineralization of BZF is enhanced by the addition of catalysts (AC and MWCNT). Successive experimental runs of BZF degradation performed with MWCNT showed that the catalyst suffers some deactivation, as a result of the introduction of oxygenated groups on the surface. Nevertheless, the catalyst remains active after several successive runs.

Compounds 3-[(4-chlorophenyl)formamido]propanoic acid (BBR), 4-chlorobenzoic acid (pCBA) and 4-chloro-N-[2-(4-hydroxyphenyl)ethyl]benzamide (BBV) are three primary products of single and catalytic ozonation of bezafibrate. Similarly to BZF, these compounds are also easily oxidized by ozone. The formation of refractory final oxidation products, such as oxalic, oxamic and pyruvic acids was also observed. The first two compounds are the most persistent carboxylic acids in solution, which hinders to attain higher mineralization degrees within the total time defined for the kinetic experiments.

The chlorine in the bezafibrate molecule is completely converted to chloride. On the other hand, the original nitrogen of the molecule is converted in all cases to  $\text{NO}_3^-$  along with smaller amounts of  $\text{NO}_2^-$  and  $\text{NH}_4^+$ . In addition, a significant part of nitrogen is converted to refractory organic by-products, such as oxamic acid and other non-identified compounds.

The presence of the radical scavenger *tert*-butanol during catalytic and single ozonation evidenced the participation of the  $\text{HO}^\bullet$  radical in the mechanism, especially contributing to the mineralization of several intermediates.

The time course of oxidation intermediaries and by-products was evaluated in order to propose a reaction sequence for the mineralization of BZF. Significantly better results were obtained in the presence of catalysts.

Microtox tests revealed that in the early stages of both single and catalytic ozonation (first 30 min) intermediaries with higher acute toxicity than bezafibrate are produced. However, for longer reaction times the acute toxicity decreases.

MWCNTs present a higher catalytic performance than activated carbon for the mineralization of bezafibrate, which may be justified by their differences in surface chemistry and by the higher internal mass transfer resistances expected for activated carbons (essentially microporous). On the other hand, the adsorption on the surface of activated carbon has a strong contribution to the removal of the bezafibrate.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.apcatb.2013.03.034>.

## References

- [1] C.G. Daughton, T.A. Ternes, *Environmental Health Perspectives* 107 (Suppl. 6) (1999) 907–938.
- [2] M. Stumpf, T.A. Ternes, K. Haberer, P. Seel, W. Baumann, *Vom Wasser* 86 (1996) 291–303.
- [3] T.A. Ternes, *Water Research* 32 (1998) 3245–3260.
- [4] D. Calamari, E. Zuccato, S. Castiglioni, R. Bagnati, R. Fanelli, *Environmental Science and Technology* 37 (2003) 1241–1248.
- [5] B. Razavi, W. Song, W.J. Cooper, J. Greaves, J. Jeong, *Journal of Physical Chemistry A* 113 (2009) 1287–1294.
- [6] J.E. Drewes, T. Heberer, K. Reddersen, *Water Science and Technology* 46 (3) (2002) 73–80.
- [7] T. Heberer, *Journal of Hydrology* 266 (2002) 175–189.
- [8] A.G. Trovó, S.A.S. Melo, R.F.P. Nogueira, *Journal of Photochemistry and Photobiology A: Chemistry* 198 (2008) 215–220.
- [9] M. Cermola, M. DellaGreca, M.R. Iesce, L. Previtera, M. Rubino, F. Temussi, M. Brigante, *Environmental Chemistry Letters* 3 (2005) 43–47.
- [10] R.F. Dantas, M. Canterino, R. Marotta, C. Sans, S. Esplugas, R. Andreozzi, *Water Research* 41 (2007) 2525–2532.
- [11] H. Yuan, Y. Zhang, X. Zhou, *Clean – Soil Air Water* 40 (2012) 239–245.
- [12] P.C.C. Faria, J.J.M. Órfão, M.F.R. Pereira, *Water Research* 39 (2005) 1461–1470.
- [13] A.G. Gonçalves, J.L. Figueiredo, J.J.M. Órfão, M.F.R. Pereira, *Carbon* 48 (2010) 4369–4381.
- [14] J.L. Figueiredo, M.F.R. Pereira, M.M.A. Freitas, J.J.M. Órfão, *Carbon* 37 (1999) 1379–1389.
- [15] J.L. Figueiredo, M.F.R. Pereira, M.M.A. Freitas, J.J.M. Órfão, *Industrial and Engineering Chemistry Research* 46 (2007) 4110–4115.
- [16] F.J. Béltran, F.J. Rivas, L.A. Fernandez, P.M. Alvarez, R. Montero-de-Espinosa, *Industrial and Engineering Chemistry Research* 41 (2002) 6510–6517.
- [17] DIN, Water quality – determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test), Part 3: method using freeze-dried bacteria, 2005.
- [18] F. Rodríguez-Reinoso, *Carbon* 36 (1998) 159–175.
- [19] N. Grobert, *Materials Today* 10 (2007) 28–35.
- [20] A. Hirsch, *Angewandte Chemie International Edition* 41 (2002) 1853–1859.
- [21] A.G. Gonçalves, J.J.M. Órfão, M.F.R. Pereira, *Journal of Hazardous Materials* 239/240 (2012) 167–174.
- [22] P. Calza, C. Medana, M. Pazzi, C. Baiocchi, E. Pelizzetti, *Applied Catalysis B* 53 (2004) 63–69.
- [23] K. Nohara, H. Hidaka, E. Pelizzetti, N. Serpone, *Catalysis Letters* 36 (1996) 115–118.
- [24] V. Maurino, C. Minero, E. Pelizzetti, P. Piccinini, N. Serpone, H. Hidaka, *Journal of Photochemistry and Photobiology A: Chemistry* 109 (1997) 171–176.
- [25] POSEIDON, <http://www.eu-poseidon.com>, POSEIDON – Contract No. EVK1-CT-2000-00047, Report: Assessment of Technologies for the Removal of Pharmaceuticals and Personal Care Products in Sewage and Drinking Water Facilities to Improve the Indirect Potable Water Reuse, 2004.
- [26] Y. Yamamoto, E. Niki, H. Shiokawa, Y. Kamiya, *Journal of Organic Chemistry* 44 (1979) 2137–2142.